REFERENCES
2. González C; Martín T; Arroyo T; García-Isidoro M; Navajo JA; González-Buitrago JM. Comparison and variation of different methodologies for the detection of autoantibodies to nuclear antigens (ANA). J Clin Lab Anal 1997;11(6):388-92.

WARNING

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INTENDED USE
The ANA Screen ELISA test system is an enzyme-linked immunosorbent assay (ELISA) for the detection of IgG class antibodies to ANA in human serum or plasma. For research use only.

SUMMARY AND EXPLANATION
Antinuclear antibodies (ANA) are frequently present in patients with systemic lupus erythematosus (SLE) and, less commonly, in other autoimmune diseases Rheumatoid arthritis, Collagen vascular diseases, chronic liver diseases and systemic sclerosis (scleroderma). ANA bind to several nuclear antigens including DsDNA, SSDNA, RNP, Sm, SSA and SSB. ANA frequency increases with age in apparently healthy people, especially women after the age of 45 years. ANA ELISA is widely used as a screening procedure for different autoimmune diseases.

PRINCIPLE OF THE TEST
Diluted patient serum is added to wells coated with purified nuclear antigens. ANA IgG specific antibody, if present, binds to the antigen. All unbound materials are washed away and the enzyme conjugate is added to bind to the antibody-antigen complex, if present. Excess enzyme conjugate is washed off and substrate is added. The plate is incubated to allow the hydrolysis of the substrate by the enzyme. The intensity of the color generated is proportional to the amount of IgG specific antibody in the sample.

MATERIALS PROVIDED

<table>
<thead>
<tr>
<th>MATERIALS PROVIDED</th>
<th>96 tests</th>
</tr>
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<tbody>
<tr>
<td>1. Microwell coated with nuclear antigens</td>
<td>12x6x1</td>
</tr>
<tr>
<td>2. Sample Diluent: 1 bottle (ready to use)</td>
<td>22 ml</td>
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<tr>
<td>3. Calibrator 1 Vial (ready to use)</td>
<td>1.5 ml</td>
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<tr>
<td>4. Positive Control 1 vial (ready to use)</td>
<td>1.5 ml</td>
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<tr>
<td>5. Negative Control 1 vial (ready to use)</td>
<td>1.5 ml</td>
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<tr>
<td>6. Enzyme conjugate: 1 bottle (ready to use)</td>
<td>12 ml</td>
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<tr>
<td>7. TMB Substrate: 1 bottle (ready to use)</td>
<td>12 ml</td>
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<tr>
<td>8. Stop Solution: 1 bottle (ready to use)</td>
<td>12 ml</td>
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<tr>
<td>9. Wash concentrate 20X: 1 bottle</td>
<td>25 ml</td>
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MATERIALS NOT PROVIDED
1. Distilled or deionized water
2. Precision pipettes
3. Disposable pipette tips
4. ELISA reader capable of reading absorbance at 450 nm
5. Absorbance paper or paper towel
6. Graph paper

STORAGE AND STABILITY
1. Store the kit at 2 – 8° C.
2. Keep microwells sealed in a dry bag with desiccants.
3. The reagents are stable until expiration of the kit.
4. Do not expose test reagents to heat, sun or strong light.
WARNINGS AND PRECAUTIONS
1. Potential biohazardous materials:
   The calibrator and controls contain human source components, which have been tested and found non-reactive
   for hepatitis B surface antigen as well as HIV antibody with FDA licensed reagents. However, as there is no
   test method that can offer complete assurance that HIV, Hepatitis B virus or other infectious agents are absent,
   these reagents should be handled at the Biosafety Level 2, as recommended in the Centers for Disease
   Control/National Institutes of Health manual, "Biosafety in Microbiological and Biomedical Laboratories." 1984.
2. This kit is designed for research use only.
3. Optimal results will be obtained by strict adherence to the test protocol. Precise pipetting as well as following
   the exact time and temperature requirements is essential.
4. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are
   handled.
5. The components in this kit are intended for use as an integral unit. The components of different lots should not
   be mixed.
6. This product contains components preserved with sodium azide. Sodium azide may react with lead and copper
   plumbing to form explosive metal azide. On disposal, flush with a large volume of water.

SPECIMEN COLLECTION AND HANDLING
1. Collect blood specimens and separate the serum.
2. Specimens may be refrigerated at 2-8 °C for up to seven days or frozen for up to six months. Avoid repetitive
   freezing and thawing.

REAGENT PREPARATION
Prepare 1X Wash buffer by adding the contents of the bottle (25 ml, 20X) to 475 ml of distilled or deionized water.
Store at room temperature (18-26 °C).

ASSAY PROCEDURE
Bring all specimens and kit reagents to room temperature (18-26 °C) and gently mix.
1. Place the desired number of coated strips into the holder.
2. Negative control, positive control, and calibrator are ready to use. Prepare 1:21 dilution of test samples, by
   adding 10 µL of the sample to 200 µL of sample diluent. Mix well.
3. Dispense 100 µL of diluted sera, calibrator and controls into the appropriate wells. For the reagent blank,
   dispense 100µL sample diluent in 1A well position. Tap the holder to remove air bubbles from the liquid and
   mix well. Incubate for 20 minutes at room temperature.
4. Remove liquid from all wells. Wash wells three times with 300 µL of 1X wash buffer. Blot on absorbance paper
   or paper towel.
5. Dispense 100 µL of enzyme conjugate to each well and incubate for 20 minutes at room temperature.
6. Remove enzyme conjugate from all wells. Wash wells three times with 300 µL of 1X wash buffer. Blot on
   absorbance paper or paper towel.
7. Dispense 100 µL of TMB substrate and incubate for 10 minutes at room temperature.
8. Add 100 µL of stop solution.
9. Read O.D. at 450 nm using ELISA reader within 15 min. A dual wavelength is recommended with reference
   filter of 600-650 nm.

CALCULATION OF RESULTS
1. Check Calibrator Factor (CF) value on the calibrator bottle. This value might vary from lot to lot. Make sure you
   check the value on every kit.
2. Calculate the cut-off value: Calibrator OD x Calibrator Factor (CF).
3. Calculate the Ab (Antibody) Index of each determination by dividing the O.D. value of each sample by cut-off
   value.

Example of typical results:
Calibrator mean OD = 0.8
Calibrator Factor (CF) = 0.5
Cut-off Value = 0.8 x 0.5 = 0.400
Positive control O.D. = 1.2
Ab Index = 1.2 / 0.4 = 3
Patient sample O.D. = 1.6
Ab Index = 1.6 / 0.4 = 4.0

QUALITY CONTROL
The test run may be considered valid provided the following criteria are met:
1. If the O.D. of the Calibrator should be greater than 0.250.
2. The Ab index for Negative control should be less than 0.9.
3. The Ab index for Positive control should be greater than 1.2.

INTERPRETATION
The following is intended as a guide to interpretation of ANA IgG test results; each laboratory is encouraged to
establish its own criteria for test interpretation based on sample populations encountered.

Antibody Index Interpretation
<0.9 No detectable ANA IgG by ELISA.
0.9-1.1 Borderline positive. Follow-up testing is recommended if clinically indicated.
>1.1 Detectable ANA IgG by ELISA.

LIMITATIONS OF THE TEST
1. The test results obtained using this kit are for research use only and are not intended to be used as a part of
   any official diagnosis.
2. Lipemic or hemolyzed samples may cause erroneous results.

PERFORMANCE CHARACTERISTICS
1. Sensitivity and Specificity
   354 patient sera were tested by this ELISA and a reference ELISA methods. 148 sera were positive and 188
   sera were negative by both methods. The agreement between the two methods was 95% (336/354).
   The test results obtained using this kit are for research use only and are not intended to be used as a part of
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2. Precision
   Intra-Assay Study
   Serum | No. of Replicates | Mean | Standard Deviation | Coefficient of Variation %
   1     | 16             | 1.41 | 0.06              | 4.22              |
   2     | 16             | 0.62 | 0.02              | 2.6               |
   3     | 16             | 0.29 | 0.03              | 9.48              |

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Inter-Assay Study
   Serum | No. of Replicates | Mean | Standard Deviation | Coefficient of Variation %
   1     | 10             | 1.15 | 0.09              | 7.43              |
   2     | 10             | 0.81 | 0.1               | 11.8              |
   3     | 10             | 0.29 | 0.03              | 9.48              |